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Multimodal Regulation of Circadian Glucocorticoid Rhythm by Central and Adrenal Clocks

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Adrenal glucocorticoids (GCs) control a wide range of physiological processes, including metabolism, cardiovascular and pulmonary activities, immune and inflammatory responses, and various brain functions. During stress responses, GCs are secreted through activation of the hypothalamic–pituitary–adrenal axis, whereas circulating GC levels in unstressed states follow a robust circadian oscillation with a peak around the onset of the active period of a day. A recent advance in chronobiological research has revealed that multiple regulatory mechanisms, along with classical neuroendocrine regulation, underlie this GC circadian rhythm. The hierarchically organized circadian system, with a central pacemaker in the suprachiasmatic nucleus of the hypothalamus and local oscillators in peripheral tissues, including the adrenal gland, mediates periodicities in physiological processes in mammals. In this review, we primarily focus on our understanding of the circadian regulation of adrenal GC rhythm, with particular attention to the cooperative actions of the suprachiasmatic nucleus central and adrenal local clocks, and the clinical implications of this rhythm in human diseases.

Freeform/Key Words: adrenal gland, circadian clock, circadian rhythm, glucocorticoid, hypothalamic-pituitary-adrenal axis

Circadian rhythm refers to evolutionarily conserved biological oscillations with a ~24-hour period. This type of daily rhythm is not a simple response to alternations of day and night; rather, it arises from a genetically operated timekeeping system called the "circadian clock." This internal timekeeping system allows organisms to anticipate environmental cycling and thus optimize their physiology, metabolism, and behavior at the right time of day. The circadian clock is cell-autonomous and self-sustainable because of the cooperation of genetic components, but it can also be entrained by external time cues called "zeitgebers." Most cells in multicellular organisms have their own cell-autonomous oscillators, which are hierarchically organized into a circadian timing system. The suprachiasmatic nucleus (SCN) of the hypothalamus, composed of densely packed neurons, generates self-sustaining rhythms by both genetic and neural mechanisms and is thus considered the central or master clock [1]. The SCN central clock receives environmental time information (primarily light) to adjust or entrain its phases. The SCN clock also orchestrates other oscillators in extra-SCN brain regions and peripheral tissues (referred to as local or peripheral clocks) to produce overt

Abbreviations: ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; BMAL1, brain muscle aryl hydrocarbon receptor nuclear translocator-like; CFS, chronic fatigue syndrome; CLOCK, circadian locomotor output cycle kaput; CRH, corticotropin-releasing hormone; CRY, cryptochrome; GC, glucocorticoid; HPA, hypothalamic–pituitary–adrenal; mRNA, messenger RNA; PER, period; PVN, paraventricular nucleus; ROR, retinoic acid receptor-related orphan nuclear receptor; SCN, suprachiasmatic nucleus; StAR, steroidogenic acute regulatory protein; ZF, zona fasiculata; ZG, zona glomerulosa; ZR, zona reticularis.

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circadian rhythms, such as the rest–activity cycle, periodic daily variations in metabolism and body temperature, and rhythmic secretion of hormones [2].

Glucocorticoids (GCs)—primarily cortisol in primates and corticosterone in rodents—are steroid hormones secreted by the adrenocortical steroidogenic cells that constitute the stress-responsive neuroendocrine system [3–5]. Threatening stimuli from the environment cause an immediate increase in the levels of circulating GCs by activating the hypothalamic–pituitary–adrenal (HPA) neuroendocrine axis. Activation of stress-sensitive neural circuits in discrete brain regions leads to excitation of neurosecretory neurons in the paraventricular nucleus (PVN) of the hypothalamus, which in turn results in the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into the hypothalamo–hypophyseal portal vessels. In turn, these secretagogues induce adrenocorticotropic hormone (ACTH) secretion from the pituitary corticotropes, which activates adrenal synthesis and the secretion of GCs. Finally, GCs interact with intracellular receptors in target cells and regulate diverse physiologic events by either genomic or nongenomic mechanisms to cope with stress. Notably, circulating GC levels oscillate robustly even in undisturbed states; that is, they exhibit clear circadian rhythm with a peak around the onset of the active period (night for nocturnal animals and day for diurnal animals) [3–5]. It is widely accepted that the daily GC rhythm is under the control of the circadian clock because the GC rhythmic profiles are completely eliminated by abolition of the SCN central clock or genetic components of the clock [6–9]. Recent studies on the adrenal clock imply a role of this local oscillator along with the SCN central pacemaker in regulating circadian GC oscillations [10, 11]. In view of these facts, the present review mainly focuses on our understanding of the circadian regulation of adrenal GC rhythm, particularly by the cooperative actions of the SCN central and adrenal clocks. Implications of adrenal GC rhythm with regard to human health and disease are also reviewed.

1. Molecular Clock and Circadian Timing System in Mammals

The intrinsic and self-sustainable nature of the circadian system is primarily attributed to the molecular oscillator driven by genetic components. The mammalian molecular clock is currently understood to be a molecular feedback loop formed by a group of clock proteins [2]. The clock proteins form two interlocked positive and negative transcription/translation feedback loops that promote periodic gene expression [Fig. 1(a)]. The foremost regulators of the core loop of the molecular oscillator belong to the basic helix-loop-helix–period (PER)-ARNT-SIM family of transcription factors and include circadian locomotor output cycle kaput (CLOCK) and brain muscle aryl hydrocarbon receptor nuclear translocator-like (BMAL1). CLOCK and BMAL1 form heterodimers, bind to E/E′-boxes (5′-CANNTG-3′) elements, and then induce transcription of the target genes through epigenetic activation of their promoters. These downstream genes include PERs (PER1–3) and cryptochromes (CRY) 1 and 2 constituting the negative limb of the feedback loop. Accumulated PER and CRY proteins form repressive protein complexes that inhibit the E-box–mediated transcription by directly binding with the CLOCK/BMAL1 complex [12–15]. In addition to the core feedback loop, the levels of CLOCK and BMAL1 proteins are also adjusted by an auxiliary or stabilizing feedback loop consisting of sets of the circadian nuclear receptors, such as retinoic acid receptor–related orphan nuclear receptors (ROR) α, β, and γ and REV-ERBα and β [16–18]. Taken together, these two interlocked feedback loops provide a molecular basis for self-sustaining circadian oscillations with a period of approximately 24 hours.

The hypothalamic SCN, considered to be the master clock that drives and organizes rhythmic physiology and behaviors, receives environmental information to adjust the circadian system to geophysical time [6, 19, 20]. Most cells in peripheral tissues and extra-SCN brain regions also contain their own oscillators that have similar molecular makeup to that found in the SCN pacemaker neurons. Therefore, the mammalian circadian system is organized in a hierarchical fashion consisting of the SCN central clock and various subsidiary local clocks. In the absence of synchronizing cues from the SCN, local clocks tend to lose phase coherence among cells and subsequently exhibit dampened rhythms [21, 22]. The SCN

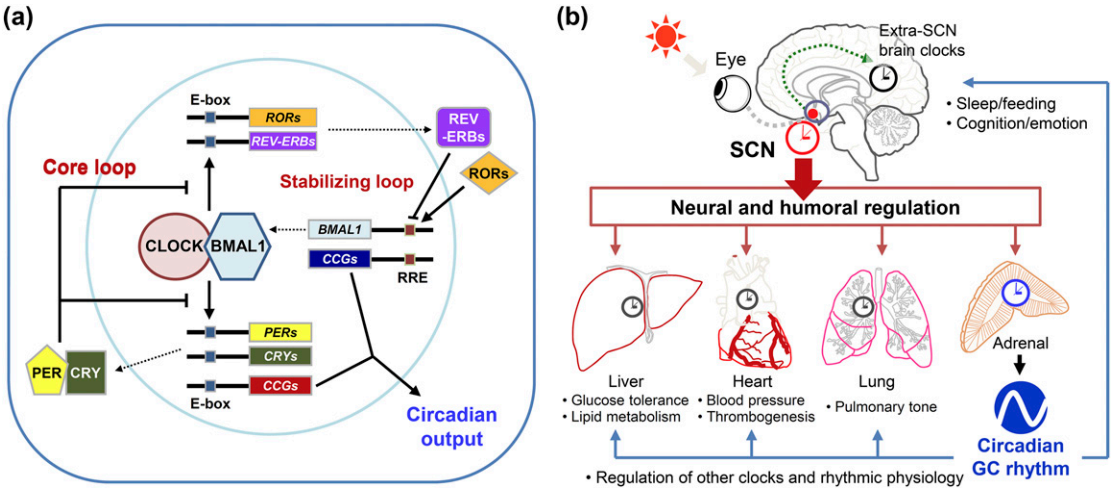

Figure 1. Schematic representations of the mammalian circadian timing system. (a) Circadian molecular clock in mammals. The mammalian circadian oscillator is composed of two interlocking transcription/translation feedback loops, designated as core and auxiliary loops, to produce precise rhythms of cyclic gene expression. (b) Hierarchical organization of the mammalian circadian system. The SCN functions as the master pacemaker responsible for the coordination of multiple clock networks throughout the body. Local oscillators in extra-SCN brain regions and peripheral tissues, in turn, constitute tissue-specific physiological outputs. Of particular interest, circadian rhythm in adrenal GCs contributes to coordination of central and peripheral rhythms.

harmonizes and coordinates the local oscillators by continuously communicating with them through various humoral and neural synchronization signals [Fig. 1(b)] [2–5].

Adrenal GCs are one of the most potent humoral links between the SCN and the periphery as demonstrated by their clock-resetting effects on a variety of peripheral oscillators [23, 24] and profound effects on diverse physiological processes [5, 25]. GC signaling is a well-established extracellular stimulus that synchronizes and promotes the cyclic expression of clock gene messenger RNA (mRNA) transcripts in various types of cultured cells [23, 26]. Administration of synthetic GC has also been shown to promote phase shifts of circadian clock gene expression in various peripheral tissues *in vivo* [23, 27]. Alternatively, accumulating evidence suggests that there are stabilizing effects of GCs on established physiological rhythms *in vivo*; for example, GCs retard the daytime feeding-induced phase shift of peripheral oscillators [27]. Ablation of the entire adrenal gland or the adrenal clock facilitates re-entrainment of the SCN-driven behavioral rhythm to a shifted light–dark cycle in a zeitgeber time-dependent manner [28, 29]. Furthermore, the circadian GC rhythm may contribute to overt rhythms by directly producing the rhythmic physiological outputs from other tissues in a more direct fashion via either classical or nongenomic GC signaling. This notion is supported by findings that the rhythmic expression of many liver genes is more dependent on the adrenal gland and GC signaling than on the hepatic oscillator [30, 31]. The periodic clock gene expression in certain brain regions also requires rhythmic GC signaling, implying that even cognition and emotion can be influenced by the adrenal rhythm [32–34]. Overall, the circulating GC rhythm has a harmonizing role in the circadian rhythms of physiology, metabolism, and behavior by synchronizing local oscillators and/or directly driving rhythmic expression of wide spectrum of GC-responsive genes in the target tissues.

2. Adrenal Peripheral Clock and Steroid Biosynthesis

The adrenal gland is composed of the cortex and medulla, which differ in terms of cell types and secreted factors [4, 5]. The adrenal medulla contains chromaffin cells, which originate from neuronal ganglionic cells and secrete epinephrine and norepinephrine. In contrast, the

adrenal cortex mainly produces steroid hormones and is divided into three separate zones [zona glomerulosa (ZG), zona fasiculata (ZF), and zona reticularis (ZR)]. Different sets of steroidogenic genes are expressed in each zone to produce different kinds of steroids from cholesterol, which is the common precursor of steroids. GCs are mainly produced by ACTH receptor–expressing ZF and ZR cells. Aldosterone, a major mineralocorticoid, and the adrenal androgens are synthesized in steroidogenic cells of the ZG and ZR, respectively.

The adrenal gland has its own functional circadian clockwork that is characterized by a cell-autonomous and self-sustaining nature [8, 10, 35–38]. The adrenal gland clock gene expression appears to be independent of the stress-responsive humoral inputs of the HPA axis [39], but it can be entrained by activation of the autonomic SCN–adrenal pathway [40]. Similar to other peripheral tissues, adrenal gland explant cultures maintain circadian oscillations in the murine *Per2* promoter-driven luciferase reporter activities [41, 42]. More importantly, numerous adrenal genes constituting a variety of cellular pathways exhibit cyclic mRNA accumulation throughout the course of the day [36–38, 40]. Microarray analyses of the adrenal gland in two independent studies revealed that ~4% to 7% of gene transcripts follow circadian oscillations in their expression [37, 38]. Comparison of these two studies, following re-evaluation by equivalent criteria using a nonparametric algorithm (JTK_CYCLE) to detect rhythmic components [43], yielded ~240 common genes that exhibit statistically significant circadian expression in both studies [Fig. 2(a) and 2(b); see Supplemental Table 1 for the full list of common rhythmic genes]. Many of the common RNA species with circadian expression are adrenal gland–enriched genes [Fig. 2(c)], implying possible crosstalk between circadian clockwork and cell type–specific transcriptional regulators in the adrenal

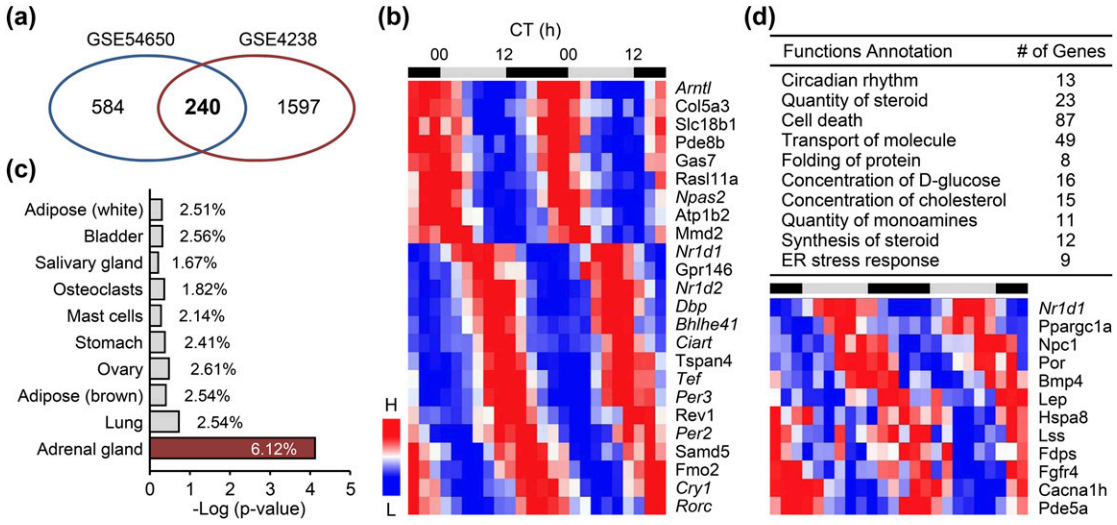

Figure 2. Genome-wide circadian rhythmicity in the adrenal gland. Circadian gene expression profiles in the adrenal gland from two independent microarray studies [37, 38] are compared. Raw data are available from the Gene Expression Omnibus database (accession nos. GSE4238 and GSE54650, respectively). (a) Venn diagram for circadian genes identified using JTK_CYCLE [43]. Rhythmic gene transcripts are defined as follows: adjusted $P < 0.05$ for rhythmicity and amplitude >10% of mean expression level of a given gene as calculated by the JTK_CYCLE algorithm. (b) Top 30 circadian genes according to P value and their expression profiles are expressed as a heat map. (c) Tissue enrichment analysis of 240 common circadian genes using the BioGPS database (http://biogps.org). The commonly rhythmic genes overlap with 6.12% of the adrenal-enriched genes with statistical significance ($P < 0.05$ for overlapping). (d) Top 10 biological functions annotated by gene set enrichment analysis using Ingenuity Pathway Analysis (https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) for the commonly rhythmic genes (upper). A heat map representation for cyclic expression profiles of the genes involved in synthesis of steroid is shown (lower). Raw data from GSE54650 [38] were used to construct heat maps in (b) and (d).

gland, similarly to that in other tissues [38, 44, 45]. Subsequent gene enrichment analysis suggests that these circadian gene transcripts are significantly linked with several biological processes, such as circadian rhythm, steroid biosynthesis, concentration of cholesterol, protein folding, and quantity of monoamine [Fig. 2(d); Supplemental Table 2].

Among adrenal genes involved in steroidogenic processes, steroidogenic acute regulatory protein (StAR) is worthy of being highlighted. Delivery of free cholesterol to the inner mitochondrial membrane, the first and rate-limiting step of GC biosynthesis, is mediated by StAR and its expression and activity are correlated with steroid biosynthesis [46]. Induced StAR expression by tropic hormones such as ACTH enhances adrenal steroidogenesis, whereas defects in StAR expression result in a dramatic decrease in steroid production [46–49]. The diurnal rhythm of adrenal StAR gene expression is quite controversial. Several reports, including our previous studies, demonstrated rhythmic StAR expression in rodent models [10, 35, 50–52]. In contrast, some microarray datasets, including examples in Fig. 2, failed to show significant rhythmicity of adrenal StAR mRNA expression [37, 38, 53]. This discrepancy may originate from the relatively modest rhythmicity in adrenal StAR expression compared with that of canonical clock genes. Nevertheless, the direct link between StAR gene transcription and the circadian molecular clock has been strongly supported by a number of independent studies. Adrenal StAR expression is reduced in mouse models with global or adrenal-specific ablation of BMAL1 expression [9, 10, 41]. In a previous study, we clearly demonstrated that StAR gene expression is transcriptionally regulated by the binding of the CLOCK/BMAL1 heterodimer to the E-box elements on its distal promoter and, more importantly, StAR gene expression mediates molecular clock–evoked steroid production in cultured adrenocortical cells [10]. The transcriptional mechanism of StAR gene expression by clock components appears to be evolutionarily conserved in both avian and mammalian reproductive systems [54, 55], supporting the role of StAR as a key molecular link between the circadian clock and steroidogenic pathway.

3. Integrative Mechanisms Controlling the Circadian GC Rhythm

A robust daily rhythm in the unstressed state and a prompt response to stress are key characteristics of GCs. Recent advances in chronobiology reveal that multiple regulatory mechanisms involved in both SCN central and adrenal-intrinsic clocks cooperate to generate the robust circadian GC rhythm in circulation (Fig. 3).

A. Regulation of the HPA Axis Activity by the SCN Central Clock

Circadian GC secretion has been primarily attributed to the SCN control of the HPA axis [56]. Ablation of the SCN completely eliminates the daily rhythms of both ACTH and GCs in circulation [7, 19]. Notably, SCN graft transplantation into SCN-lesioned hamsters restores circadian rhythmicity in locomotion, but not GC rhythm, indicating that synaptic connectivity may underlie SCN control of the HPA axis [57]. From a neuroanatomical perspective, SCN pacemaker neurons appear to indirectly control ACTH secretagogues by innervating neighboring neurons in the subparaventricular zone and the dorsomedial hypothalamus nucleus, which are in turn connected to the CRH/AVP neurons in the PVN [58, 59]. Given that trough corticosterone levels have been reported to increase after the SCN lesion in rats, the regulation of basal GC release by the SCN seems to be inhibitory [7]. The AVP produced by a subset of SCN neurons is considered one of the main neurotransmitters mediating this inhibition [60, 61]. Another possibility involves the presence of local clockworks in the PVN and pituitary [35, 62]. The local rhythms in ACTH secretagogue-producing neurons in the PVN and pituitary corticotropes may have a certain role in the adrenal GC rhythm.

However, several lines of evidence suggest relatively restricted roles for the upstream hormonal regulators of the HPA axis and the involvement of multiple inputs into the adrenal gland. First, circulating ACTH shows a circadian profile similar to that of circulating GC rhythm but usually has relatively lower amplitude than that of the GC rhythm [10, 63–65].

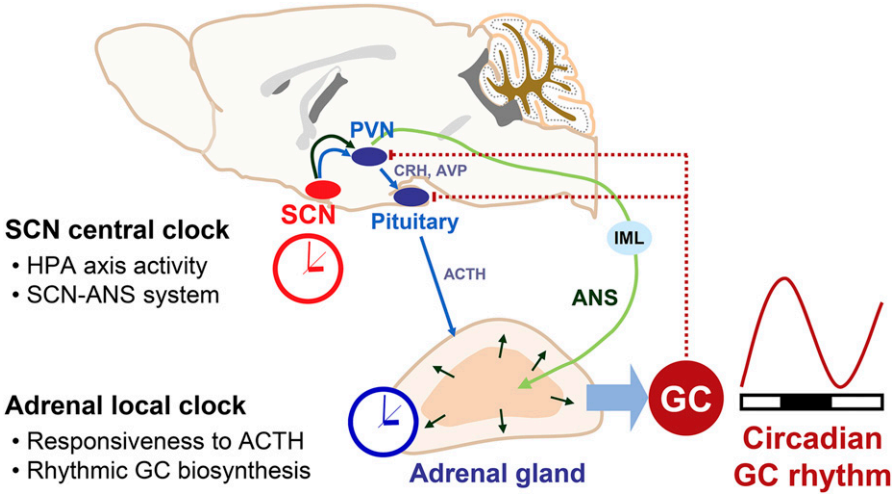

Figure 3. Cooperative actions of central and adrenal clocks underlying circadian GC rhythm. The robust daily variations in circulating GC levels are achieved by multiple regulatory mechanisms. The SCN central clock regulates the adrenal rhythm by modulating the HPA axis as well as through autonomic neural inputs [autonomic nervous system (ANS)] into the gland. In addition to the central mechanisms, adrenal-intrinsic mechanisms involving the adrenal local clock also underlie the circadian GC rhythm. The adrenal peripheral clock gates adrenal sensitivity to ACTH and controls the GC biosynthesis through coordinated transcriptional regulation of a subset of adrenal steroidogenic genes.

Furthermore, the circadian rhythm of CRH does not account for rhythms observed in its downstream targets [35, 63, 66], suggesting that additional regulatory activity is required for the robust adrenal rhythm. Second, the rhythmic release of CRH/ACTH may not be essential for circadian GC rhythm; the plasma GC rhythm persists even in hypophysectomized rats with implanted ACTH pellets [67, 68]. Similarly, a constant infusion of CRH was sufficient to rescue diurnal GC rhythm in CRH knockout mice [69]. Also note that even in the absence of SCN inhibitory signals, plasma GC levels did not reach a peak level, and there was an apparent discrepancy in rats between the decrease in inhibitory regulation of the SCN and the afternoon increase in circulating GC levels [7, 70]. Based on a series of disinhibition experiments, Buijs and colleagues [70] suggested a requirement for additional stimulatory signals, with a delay of several hours after the major inhibitory signal but preceding the peak GC level.

B. Regulation of Daily GC Rhythm by the SCN-Autonomic System

Circadian GC rhythm is maintained in hypophysectomized animals with a constant supply of ACTH, whereas adrenal rhythm is completely abolished in hypophysectomized rats after denervation of the adrenal glands, showing the involvement of a neural mechanism [68]. In this regard, several studies suggest the impact of the SCN central pacemaker via splanchnic nerve innervation of the adrenal gland [40, 61, 71–73]. SCN-derived autonomic nervous system signals can be transmitted through preautonomic PVN neurons and sympathetic preganglionic intermediolateral neurons of the spinal cord to reach the adrenal gland [61]. One possible explanation for autonomic control of the daily GC rhythm involves the regulation of adrenal sensitivity to ACTH. The adrenal responsiveness to ACTH follows a diurnal rhythm, with a higher sensitivity during the active phase in rodents [74], and such daily variation depends on an intact SCN and splanchnic innervation of the adrenal glands [71, 72, 75]. Another significant feature of the SCN-driven neural pathway is that sympathetic innervation provides a rapid photic input to the gland, leading to increased GC release in mice during subjective night regardless of the state of HPA axis activation [40]. The light pulses activate the sympathetic nervous system in an SCN-dependent fashion, and adrenal

denervation abolishes the light-induced GC release [40, 76]. Release of GCs in response to a brief light pulse occurs in a delayed fashion compared with stress-evoked GC secretion, supporting the involvement of a pathway other than the HPA axis [40], which is independent of GC synthesis and of the local clock in the adrenocortical GC-producing cells [11]. Medullary–cortical signaling pathways have been proposed as mediators of sympathetic nerve-dependent adrenal activation via catecholamines, neuropeptides, and intra-adrenal blood flow [77].

We have recently shown that photic signal-evoked GC secretion is associated with decreased adrenal GC content, suggesting neural mechanisms acutely release the steroids from the stored pool [11]. It has long been thought that steroid hormone secretion is primarily controlled by the regulation of steroidogenesis through concentration gradient–dependent diffusion. However, several studies observed the retention of steroids against a concentration gradient at intracellular sites proximal to the plasma membrane, and they proposed a possible steroid transport mechanism involving organic anion transport [78, 79]. More recently, it has been suggested that acute release of stored GC can be induced by stress, and this release may be mediated by the paracrine actions of prostaglandins and subsequent production of nitric oxide [80]. Ultrastructural analysis revealed more microcytotic vesicles and filopodia-like structures of the cell membrane in adrenocortical cells exposed to stress than in unstressed cells. In activated adrenocortical cells, invaginations in close contact with mitochondria, lipid droplets, and additional microcytotic vesicles are also observed [80]. It is plausible that medullary–cortical interactions and accompanying intracellular signals leading to the rapid release of GC may mediate the SCN–autonomic system-dependent increase in circulating GC levels.

C. Adrenal-Intrinsic Mechanisms: The Role of the Adrenal Local Oscillator

In addition to the SCN-driven central mechanisms, growing evidence suggests the presence of adrenal-intrinsic mechanisms, particularly involving the adrenal local oscillator. The circadian GC rhythm is often more severely attenuated than other rhythmic physiology and behavior patterns in clock gene-deficient mice. Mice bearing a defective *Per1* or *Per2* allele exhibit normal daily cycles in locomotor activity under light–dark conditions but significantly altered daily GC rhythms [81, 82]. Restricted daytime feeding of nocturnal rodents supports the importance of the adrenal clock. The daytime feeding regimen is known to dissociate the phases of the SCN central pacemaker and other peripheral oscillators, including the adrenal clock, presumably by the action of food-entrainable oscillators in the dorsomedial hypothalamus [11, 83, 84]. Under this condition, the daily GC profile has two distinct peaks per day, strongly suggesting that the circadian GC rhythm is produced by the integrated activity of feeding-independent SCN central clock and food-entrainable local oscillators [11, 85, 86].

Based on the rhythmic expression of genes controlling ACTH receptor signaling and evidence obtained from well-designed transplantation experiments, Oster *et al.* [8, 37] proposed that the adrenal clock is involved in the circadian GC rhythm by gating adrenal sensitivity to ACTH. Splanchnic innervation entrains the adrenal local clock, and the autonomic SCN–adrenal pathway is involved in the daily variation in adrenal receptivity to ACTH [40, 59], suggesting that the adrenal clock might link the SCN neural inputs to the rhythmicity of the gland's responsiveness. However, there remains the important issue of paracrine interaction between the adrenal cortex and medulla [77]. There is a need to clarify which peripheral clock (in the adrenal cortex or medulla) plays the key role in modulating adrenal sensitivity to ACTH. Studies with mutant mice models provide some insight into this issue. Adrenal GC secretion in response to ACTH is drastically impaired in global *Bmal1*-null mice [9]. However, stress-evoked expression of ACTH and secretion of corticosterone are not significantly attenuated in mice models with impaired *Bmal1* expression in adrenocortical steroidogenic cells [10, 41]. These findings collectively indicate that the local clock in GC-producing cells may have a marginal role in controlling adrenal responsiveness to the tropic hormone.

Several lines of evidence imply a more direct role of the adrenocortical clock in the circadian regulation of GC production. For example, pioneering studies demonstrated the rhythmic nature of adrenal GC biosynthesis and secretion in isolated adrenal explants without any external cues [87, 88]. We also reported that cyclic steroid production from a cultured adrenocortical cell line was induced by a brief serum treatment, which evoked and synchronized clock gene oscillations in these cells [10]. Several rhythmic steroidogenic genes controlled by adrenal local clock, such as *Star*, *Stard4*, *Ldlr*, and *Por*, strongly suggest an adrenal-autonomous mechanism involving intrinsic GC biosynthesis [9, 10, 37]. Indeed, the levels of both adrenal and circulating GC display robust daily oscillations with similar phases to each other *in vivo* [10].

Our previous studies to dissect the adrenal and circulating levels of GC have unraveled important roles of the adrenal local clock in circadian GC rhythm. Suppression of BMAL1 expression selectively in the ACTH receptor–expressing adrenocortical cells in transgenic mice flattens the daily oscillation in adrenal GC content, whereas attenuated, but significant, circadian rhythm of GC in circulation persists [10]. Interestingly, daytime-restricted feeding that shifts the phase of the adrenal clock from the SCN in nocturnal mice produces two split peaks of circulating GC levels as mentioned above, but only shifts the peak in adrenal GC content to phases similar to those of adrenal clock genes, which is in accord with the daytime feeding-specific GC peak in circulation [11]. More importantly, the daytime feeding-specific peaks in adrenal and plasma GC can be produced even in SCN-ablated mice. This result supports adrenal local clock- and steroidogenesis-dependent regulation [11]. Nevertheless, the robust oscillation of the adrenal peripheral clock is primarily maintained by synchronizing actions of the SCN-autonomic system [40]. Furthermore, it took several days in constant darkness for the adrenal clock–suppressed mice to exhibit attenuated GC rhythms, indicating that environmental cycles may be capable of compensating defects in the adrenal peripheral clock [10]. It is therefore reasonable that under normal 24-hour light–dark cycle conditions, rhythmic GC production by the adrenal local oscillator has a supporting role, which contributes to the robust daily rhythm in the circulating GC levels by maintaining a releasable pool of the steroid hormone in a circadian fashion.

4. Deregulation of Circadian GC Rhythm Related to Various Human Diseases

Dysregulated GC secretion is related to various pathological conditions due to its impacts on a wide range of biological processes [4, 5]. Abnormalities in circadian GC rhythms are associated with human diseases, including Cushing syndrome, adrenal insufficiency, metabolic and cardiovascular diseases, and neuropsychiatric disorders [89–91]. It is often not clear whether the disrupted GC rhythm is causal to or a consequence of these diseases. Nevertheless, the importance of the GC rhythm has been well recognized in the course of therapeutic applications. For example, a tonic application with a constant dose of GC in the treatment of adrenal insufficiency does not effectively alleviate symptoms as expected, but often exacerbates cardiovascular, metabolic, and psychiatric disturbances [92]. In this section, we focus on several human diseases closely related to dysregulation of circadian GC rhythm.

A. Cushing Syndrome and Adrenal Insufficiency

Disrupted circadian rhythmicity in circulating GC is frequently found in pathological conditions associated with either GC excess or insufficiency. Cushing syndrome is a prevalent form of hypercortisolism that can be caused by either an ACTH-dependent or -independent mechanism. The long-term consequences of severe hypercortisolism include diabetes mellitus, hypertension, dyslipidemia, osteoporosis, bone fractures, recurrent infections, sleep disorder, and psychiatric abnormalities, and even mild hypercortisolism has harmful effects on long-term health resulting from decreased insulin sensitivity and altered glucose tolerance

[89, 93, 94]. Cushing syndrome patients tend to exhibit increased trough levels, an altered diurnal pattern, and an increase in circulating levels of cortisol [95]. In this context, late-night salivary cortisol measurement has been proposed as a diagnostic test for Cushing syndrome [89, 96].

The opposite condition, adrenal insufficiency, can arise from Addison disease, congenital adrenal hyperplasia with defective adrenal steroidogenesis, or certain pituitary diseases. Adrenal insufficiency is commonly characterized by vulnerability to stress, hypoglycemia, hypotension, hyperactivity in the immune/inflammatory systems, and some psychiatric symptoms [97, 98]. Although rhythmic levels of circulating cortisol are often observed in patients with adrenal insufficiency, the amplitude of the rhythms is highly attenuated by the reduction in peak GC levels [92, 98]. Both hypercortisolism and hypocortisolism are linked with disruptions of the circadian timing system, such as sleep disturbance, fatigue in the active period, affective disorders, and cognitive defects [90, 91, 99]. These common features strongly suggest that an attenuated circadian GC rhythm by itself may mediate some pathological outcomes of GC excess or insufficiency.

B. Environmental Disruption of Circadian GC Rhythm

Prolonged disturbance of intrinsic circadian rhythms by environmental factors, such as shift work, jet lag, sleep disturbance, and mistimed eating, evokes a misalignment of internal circadian clocks and external time and can have hazardous health consequences. For example, shift work is known to have long-term effects on the likelihood of onset of obesity, insulin resistance, hyperlipidemia, ischemic heart disease, and even cancer [100, 101]. Chronic jet lag may influence cognitive impairment associated with reduced temporal lobe activity of the brain [102]. It is noteworthy that the symptoms related to such chronic circadian misalignment share many features with those of human diseases associated with GC dysregulation [90, 91, 97]. Indeed, chronic disturbance of circadian rhythms results in an attenuated GC rhythm, particularly by elevating the nadir levels in both humans and rodents [103, 104], and increased cortisol levels are closely related to cognitive deficits in human subjects repeatedly exposed to jet lag [102]. It is therefore plausible that chronic disruption of circadian GC rhythm impacts the capacity to adapt to environmental disruption of the circadian system, thereby mediating pathological consequences.

C. Circadian Rhythms in the Adrenal Gland and Metabolic Diseases

One of the most important functions of circadian GC secretion appears to be a proactive regulation of energy balance for the activity phase. In agreement with this notion, an increase in the GC level usually precedes the beginning of the activity period in both diurnal and nocturnal species. Elevated circulating GC levels stimulate metabolic processes to supply an immediately available energy source [4, 5]. The importance of GCs in maintaining metabolic functions is strongly supported by symptoms of pathological GC excess and insufficiency as described above. Additionally, both experimental and clinical evidence implies that attenuated circadian GC rhythms are linked with metabolic disorders such as obesity, type 2 diabetes, dyslipidemia, and atherosclerosis [90, 105–107]. The findings strongly suggest that dampening of the GC rhythm may have a key role in the onset or progression of metabolic dysfunctions presumably by impacting metabolic tissues such as liver, skeletal muscle, adipocytes, and pancreas.

The coordinated control of hepatic carbohydrate metabolism by GC signaling and the hepatic clock has been demonstrated. Genome-wide gene expression studies clearly show that a number of the hepatic genes involved in carbohydrate metabolism exhibit diurnal variation in their expression [30, 31, 38]. Interestingly, liver-specific *Bmal1*-ablated mice exhibit metabolic abnormalities distinct from those of global $Bmal1^{-/-}$ mice, suggesting that hepatic metabolism is under the control of multiple regulatory mechanisms involving the hepatic local clockwork as well as the systemic inputs [108]. Rhythmic expression of some

hepatic genes with pivotal roles in metabolic functions, such as phosphoenolpyruvate carboxykinase 1 and carnitine palmitoyltransferase 1, is more dependent on an intact adrenal gland and/or GC signaling than on the peripheral circadian oscillator [30, 31]. Similarly, a large number of genes with circadian expression in muscular tissue were also identified as GC-responsive genes [109]. Furthermore, cyclic expression of canonical circadian clock genes in visceral adipose tissue is drastically attenuated in adrenalectomized rats, suggesting that normal molecular rhythms in adipocytes are highly dependent on circulating GC rhythm [110]. It can thus be postulated that the circulating GC rhythm may contribute to the circadian control of metabolic processes by acting on metabolic tissues either directly or indirectly through local clockworks in those tissues.

D. Circadian GC Rhythm and Psychiatric Illness

The brain is also a target of circadian GC signaling, and dysfunctions of both the circadian system and adrenal GC secretion are reported to be key risk factors for the onset of various psychiatric illnesses such as sleep disturbances, affective disorders, and cognitive impairment [42, 111]. Hyperactivity of the HPA axis by impaired negative feedback regulation is one of the well-established characteristics of patients suffering from major depressive disorder. Major depressive disorder patients usually exhibit higher circulating cortisol levels with reduced rhythm amplitude [112]. Hypercortisolism in depressed patients appears to be causal to some symptoms as indicated by a report that the unipolar depression is found in 50% to 80% of Cushing disease patients [94]. Hypocortisolism and reinforced negative feedback in the HPA axis are also associated with other forms of depression and anxiety-related disorders such as posttraumatic stress disorder [113] and chronic fatigue syndrome (CFS) [114]. Considering crucial roles of adrenal GC in processing aversive memory, dysregulated GC secretion during formation or extinction of conditioned fear could be linked with the onset and/or symptoms of posttraumatic stress disorder [115, 116]. Although it is still debatable whether disturbed HPA function is a cause or a consequence of CFS, some phenotypes of our transgenic mice with dampened GC rhythms due to adrenal-specific abolition of BMAL1 expression are comparable to symptoms observed in CFS patients [117]; that is, they exhibited hypolocomotion but a normal temperature rhythm during the activity period [10]. Alternatively, the altered circadian phase of the GC rhythm is often linked with other forms of psychiatric disorders, such as seasonal affective disorder, bipolar disorder, and schizophrenia. For example, phase-delayed cortisol and body temperature rhythms are found in seasonal affective disorder patients [118], whereas phase advances in salivary cortisol profiles as well as clock gene expression in oral mucosa are accompanied by the onset of manic episode of bipolar disorder [119]. Taken together, these findings suggest the importance of circadian GC rhythm in proper control of emotional and cognitive functions, presumably by interactions with circadian rhythms in neurotransmitter and neuromodulator systems in the brain.

5. Conclusion

In conclusion, the current understanding of the circadian control of the adrenal GC and human diseases related to disruptions of this temporal regulation indicate that GC secretion and biosynthesis are tightly controlled by coincident multimodal mechanisms. The central rhythm of the SCN directly drives the circadian GC rhythm by modulating the HPA axis and sympathetic innervation of the adrenal gland. The oscillating adrenal clock plays additional roles in maintaining the rhythm by controlling the adrenal capacity for steroidogenesis and the responsiveness to ACTH. The next question regarding circadian GC rhythm may be its relevance in physiology and pathophysiology. Although the importance of rhythmic GC profiles has been well recognized for a long time, the precise roles and modes of actions of the rhythm, particularly in human health and diseases, still remain to be further elucidated. Recent advances in our knowledge of circadian GC rhythm may provide valuable insights into its clinical applications for prognostic, diagnostic, and therapeutic purposes.

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